SOME FILAMENTOUS FUNGI TESTED FOR CELLULOSE DESTROYING POWER[1]

FREEMAN M. SCALES

Every year large quantities of cellulose in the various combinations occurring in plant tissue are returned to the soil. In this tissue there is also a great deal of carbohydrate material, such as sugar, starch, pectose, and hemicellulose. These substances are more easily available and will under ordinary circumstances be all used up before the cellulose is attacked. In spite of this abundance of easily available food, at the end of several months practically all the plant tissue will be disintegrated and split into soluble substances and humus. The destruction of this large quantity of cellulose in such a comparatively short time means that the cellulose destroying organisms must work very vigorously. By special methods of culture, cellulose dissolving bacteria have been obtained which hydrolyze this complex material very rapidly, but when plating on cellulose agar directly from a soil for the isolation of cellulose destroying organisms the filamentous fungi usually grow more abundantly and destroy more cellulose than do the bacteria. In order to determine whether the addition of cellulose to soil makes any difference in the number of molds in it, 2 per cent of cellulose was added to 200 grams of soil which was moistened to the optimum with distilled water and then incubated for 30 days at 30° C. The initial count of this soil on cellulose agar was 20,000 mold colonies. A check sample which received no cellulose, but otherwise had the same treatment as the one described, gave on the same kind of media a count of 100,000 mold colonies, while the sample which received cellulose showed a mold content of 200,000,000. It is evident from these data that the filamentous fungi are an important factor in the dissolving of cellulose in the soil.[6]

It is a well known fact that filamentous fungi are very numerous in woodland soils and also that they are abundant in acid arable

[1] Published by permission of the Secretary of Agriculture.

soils where the reaction is naturally favorable for them. MARCHAL[2] found numerous mycelia of molds in acid humus soils which were naturally rich in organic material, and he believed that they took an active part in the mineralization of organic nitrogen under these conditions, but that in an arable soil under active cultivation the molds were relatively few in number owing to the alkaline reaction and the absence of large quantities of organic matter. While the filamentous fungi are naturally thought of as occurring in great numbers in acid soils, still the recent work of DALE[3] in England indicates that in some cultivated alkaline soils they are just as numerous. JENSEN[4] at Ithaca found a great variety of filamentous fungi in alkaline cultivated soils. Lately TRAAEN[5] has reported the results of his investigation of the species and physiological activity of some of the soil fungi of Norway. In our own investigations on the destruction of cellulose by bacteria and filamentous fungi, we worked almost entirely with alkaline soil that had been under active cultivation, and found among the fungi occurring in both great variety and enormous numbers many species active cellulose destroyers. The physiological activities of these organisms on organic material, whether nitrogenous or not, are well recognized. All these data indicate that the filamentous fungi are important factors in carrying on the biological processes in cultivated soils.

In further study[6] of the destruction of cellulose by microorganisms, 19 species of cellulose destroying filamentous fungi were identified and two new ones found. One of these species was found[7] to produce a very active cytase. The present work was

[2] MARCHAL, EMILE, Sur la production de l'ammoniaque dans le sol par les microbes. Bull. Acad. Roy. Belgique III. **25**:741. 1893.

[3] DALE, ELIZABETH, On the fungi of the soil. Ann. Mycol. **10**:452–477. *pls.* 5. 1912; **12**:33–62. *pls.* 5. 1914.

[4] JENSEN, C. N., Fungous flora of the soil. N.Y. (Cornell) Agric. Exp. Sta. Bull. 315. pp. 415–501. 1912.

[5] TRAAEN, A. E., Untersuchungen über Bodenpilze aus Norwegen. Nyt. Mag. Naturv. B **52**:20–121. *pl.* 1. 1914.

[6] MCBETH, I. G., and SCALES, F. M., The destruction of cellulose by bacteria and filamentous fungi. U.S. Dept. Agric., Bur. Pl. Ind. Bull. 266. 1913.

[7] KELLERMAN, K. F., Formation of cytase by *Penicillium pinophilum*. Bur. Pl. Ind. Circ. no. 113. pp. 29–31. 1912.

undertaken for the purpose of determining more species of the filamentous fungi that are capable of exercising this function.

Dr. CHARLES THOM, mycologist of the Bureau of Chemistry, kindly supplied us with the 30 species of *Penicillium* and 10 species of *Aspergillus* that were used in this work. The cellulose destroying power of these organisms was determined with two different sources of nitrogen. An ammonium sulphate cellulose agar and a peptone cellulose agar were used for this purpose. The ammonium sulphate medium was prepared by the method described in a previous publication,[6] and the peptone medium by substituting 1 gram per liter of peptone for the ammonium sulphate.

The stock cultures were kept on Czapek's agar. The spores from fresh growths on this medium were added to freshly poured duplicate cellulose agar plates of both media. The plate cultures were inoculated in four places. Test tube cultures on both media were also made in duplicate at the same time. The Petri dishes were placed in moist chambers and then put in the incubator along with the tube cultures, which were kept at 28–30° C. for two months. They were then examined for an enzymic zone either around the colony on the plates or underneath it in the tubes. The size of this enzymic area varied considerably in the different cultures, ranging from a very thin clear zone less than 1 mm. to one 24 mm. deep in some of the tubes. Although the depth of the clear zone was fairly constant in the duplicate tubes, this phase of the work will not be emphasized until more data are obtained.

As the surface growth on cellulose agar is frequently scanty, with few definite microscopic differences, it seemed best to transplant from the plates and tubes onto Czapek's agar, in order to check each organism that destroyed cellulose against a growth on Czapek's agar made from the stock material of this species. A microscopic examination was made in each case.

The results are given in the accompanying table. The apparent failure of a number of the organisms fermenting cellulose with ammonium sulphate as the source of nitrogen to do the same with peptone as the source of nitrogen may be accounted for in two ways. One is that the organisms produced such an abundant sterile growth in the medium that any slight clearing was obscured;

the other is that the carbon in the peptone was utilized, being more easily available, and the cellulose was not attacked.

TABLE I

RESULTS OF THE TEST TO DETERMINE THE CELLULOSE DESTROYING POWER OF SOME FUNGI WITH DIFFERENT SOURCES OF NITROGEN

+ indicates cellulose destroyed; − indicates cellulose not destroyed.

Species	Ammonium sulphate	Peptone
1*a*. Penicillium luteum Zukal	+	+
2*a*. " pinophilum Hedg.	+	−
3*a*. " rugulosum Thom	+	+
4*a*. " sp. no. 2670 Thom.	+	−
5*a*. " purpurogenum O. Stoll.	+	+
6*a*. " duclauxi Dela.	+	.....
7*b*. " commune Thom.	−	−
8*b*. " biforme Thom	−	−
9*b*. " sp. no. 66 Thom.	−	−
10*b*. " sp. no. 13 Thom.	+	+
11*c*. " expansum Link.	+	+
12*c*. " sp. no. 2694 Thom	+	−
13*d*. " chrysogenum Thom.	+	+
14*d*. " notatum West	+	.....
15*d*. " sp. no. 12 Thom	+	.....
16*e*. " camemberti Thom.	−	−
17*e*. " camemberti var. rogeri Thom	−	−
18*f*. " intricatum Thom.	+	+
19*f*. Spicaria simplisissima Jensen	+	−
20. Penicillium lanosum West.	+	+
21. " claviforme Bainier.	+	+
22. " granulatum Bainier.	+	+
23. " roqueforti Thom.	+	+
24. " spinulosum Thom	+	−
25. " funiculosum Thom	+	.....
26. " lilacinum Thom	−	−
27. " divaricatum Thom	−	−
28. " sp. no. 64 Thom.	+	+
29. " sp. no. 3505 Thom.	+	−
30. Scopulariopsis repens Bainier.	+	−
31. Aspergillus candidus Link	−	−
32. " clavatus Desm.	+	−
33. " flavus Link	+	+
34. " fumigatus Fresen.	+	+
35. " nidulans Eidam	+	−
36. " niger VanTiegh	+	+
37. " oryzae Ahlb	+	−
38. " Wentii Wehmer	+	−
39. " sp. no. 144 Thom*	+	+

*Another culture of this species sent in from the Riverside Experiment Station, California, by Mr. I. G. MCBETH, gave the same reactions.

Some cultures of *Actinomyces* that have been accumulated for a classification of this group were also tested, and of 31 cultures, 8

dissolved the cellulose agar containing ammonium sulphate as a source of nitrogen. These cultures all show marked microscopic differences and are without doubt different species. These 8 organisms may be of the same species as some of the 12 cellulose destroying species which KRAINSKY[8] has recently described.

SOIL BACTERIOLOGY LABORATORY
U.S. DEPARTMENT OF AGRICULTURE

[8] KRAINSKY, A., Die Aktinomyceten und ihre Bedeutung in der Natur. Centralbl. Bakt. **41**:649–688. 1914.